DOI:10.22059/IJVM.2023.367748.1005467 Iranian Journal of Veterinary Medicine Original Article

Online ISSN: 2252-0554

The Effect of Age and Gender on Natural Calves Lung Surfactant Function as a Valuable Therapeutic Agent for Respiratory Distress Syndrome

Running Head: Effect of Age and Sex on Lung Surfactant

Marzieh Mokhber Dezfouli[1], Zohre Eftekhari[2,4*] Sirous Sadeghian Chaleshtori[3,4]

1-Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Science, Tehran-Iran

2-Biotechnology Department, Pasteur Institute of Iran, Tehran- Iran

3-Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran-Iran

4-Institute of Biomedical Research, University of Tehran, Tehran, Iran

Abstract

Background: Exogenous surfactant from natural sources helps to restore normal lung function in premature cases. Pulmonary-surfactant dysfunction can lead to acute lung injury and is characterized by alveolar instability, floating, and collapse. These abnormalities have been shown to occur in acute respiratory distress syndrome (ARDS) and neonatal respiratory distress syndrome (NRDS).

Objectives: To identify the best source of exogenous natural surfactant and its composition. **Methods:** Twenty-four healthy Holstein calves were selected in three age groups in both sexes to investigate the impact of sex and age on the surfactant composition. Cell free Bronchoalveolar lavage fluid (BALF) supernatants were centrifuged at 20000 g for 60 min, allowing separation of crude surfactant pellets (CSP). Subsequently, the supernatant was discarded, and CSP was separated into several aliquots and stored at -80 ºC for further analysis.

Results: It was concluded that BALF in female groups was significantly enriched by Surfactant proteins -C and Surfactant proteins -D in comparison with male groups at the same age. Total phospholipids, glycerides and cholesterols were not age-dependent in the male groups; however, they had a descending manner associated with age in the female groups.

Conclusions: It can be inferred that both age and sex could affect the amount of surface tension trend increased by aging and was seen to decrease in the female group compared to the male group. Female calves in the younger age group are the best source of natural surfactants required for exogenous surfactant in neonatal respiratory disease due to the highest concentration of dipalmitoylphosphatidylcholin and lowest surface tension.

***Keywords*:** Calf, Factors, Lung, Profile Analysis Technique, Respiratory Distress

1.Introduction

Lung surfactant is a complex of lipoproteins that produced, stored and secreted in the type II epithelial cells, and covered the lung alveolar epithelial surface in the last weeks of pregnancy (Choi *et al*., 2020). The component of natural surfactant includes phospholipids, cholesterol, triglyceride and four types of proteins (Guzmán and Santini, 2019).

Surfactant is composed of ~80–85% phospholipids, 5–10% neutral lipids and 8–10% protein, with 5–6% containing of the four specific surfactant proteins. Eighty-five percent of the phospholipid fraction itself contains of phosphatidylcholines, the most important component (40%) with the highest compaction properties being Dipalmitoylphosphatidylcholine (DPPC); 11% consists of phosphatidylglyerol and phoshatidylinositol, which fluidize the lipid monolayer. The remaining fraction consists of various phospholipids with particular functions (Hentschel *et al*.,2020). DPPC, the main phospholipid portion of the lung surfactant, decreases surface tension, promote compliance, and facilitate the lung to expand without complications, reducing respiratory process (Bae *et al*., 2019).

Hydrophobic surfactant proteins B and C, play a crucial role in natural surfactant structure and cause both adsorption and distribution of phospholipids at the air-liquid interface (Olmeda *et al*., 2017; Hentschel *et al*.,2020). Hydrophilic surfactant proteins A and D have a similar structure and participate in down-regulating the inflammatory response of the lung and innate immunity (Sardesai *et al*.,2017).

Neonatal respiratory distress syndrome (RDS) is one of the most common problems for preterm infants (Magni *et al*., 2023). It is common in neonates born before 27 to 32 weeks and needing ventilation with infusion exogenous lung surfactant from natural sources, helping to restore normal lung function (Han and Mallampalli, 2015; Hockenberry and Wilson, 2018). Destruction of type II alveolar epithelial cells due to ARDS (adult respiratory distress syndrome) results in an increase in lung compliance due to dysfunction in surfactant manufacture (Cutts S *et al*., 2017). Common pathogens related to ARDS which can have the effective impact on surfactant quality and its content include *Streptococcus pneumonia*, *Pneumocystis jirovecii*, *Staphylococcus aureus*, and a diversity of respiratory viruses such as H1N1 novel influenza A, novel coronavirus (COVID-19) and respiratory syncytial virus (RSV) (Al-Abedi *et al*.,2022; Ashrafi *et al*., 2020; Mojibi *et al*., 2022; Rawal, G *et al*., 2017; Jamaatia *et al*., 2020). In addition, different factors affect lung surfactant content and its maturation such as age, sex, intrinsic cortisol, thyroid

hormones, prolactin, epidermal growth factor, diabetic mother and testosterone levels (Han and Mallampalli , 2015).

The process of extracting, purifying and identification pulmonary surfactant extracted also affects the composition and phospholipid ratio (Christmann *et al*., 2006). Nielson and Torday mentioned the biological disparity between fetal sexes in the fetal rabbit as an animal model, which may be the cause for male infants to develop respiratory distress syndrome (Nielsen and Torday, 1981).

Moreover, some studies indicated that aging could also modify the composition and function of lung surfactant (Christmann *et al*., 2006). Meanwhile John Clements showed the first direct measurements of pulmonary surfactant using his home-made Langmuir–Wilhelmy surface balance half a century ago. Subsequently, many more in vitro tensiometric techniques, such as the pulsating bubble surfactometry, captive bubble surfactometry, and the constrained sessile drop, have been developed to assess in vitro surfactant function (Stichtenoth et al., 2014).

Although some researchers have been conducted on lung surfactant composition, there is still insufficient data to evaluate the effect of age and gender on lung surfactant content, especially in calves as an appropriate source of exogenous surfactant which consumes in respiratory diseases.

The main aim of this study was to determine the effect of age and sex factors on quality and quantity of lung surfactant and measure its functionality by new methods, which potentially can support in finding the best source to obtain natural surfactant for RDS (respiratory distress syndrome) or the other respiratory disorder.

2.Materials and Methods

Animals

Twenty-four healthy Holstein calves were collected from Tehran University of Medical Sciences (TUMS) farm and divided into the following three groups: 0-4months, 4-8months, and 8-12months (n=8 in both sexes in each group). All calves' characteristics such as age, weight, and gender were registered. The calves' general health was assessed through clinical exams, and blood samples were taken to assess their complete blood count and confirm their health. The clinical criteria for admission to the study included the absence of eye and nasal discharge, as well as normal body temperature and absence of respiratory sounds/cough. The calves were kept under controlled conditions for approximately 12 hours prior to the procedure (Fozouni and Tahaei 2023). All experimental procedures followed the guidelines on ethical standards for

experimental processes in animals, according to a protocol approved by the Animal Ethics Committee, University of Tehran, Iran.

Bronchoalveolar Lavage Method

Bronchoalveolar lavage (BAL) performed in the anesthetized calves with Propofol (Fresenius Kabi, U.S.A.) (Diprivan®5mg/kg) using a sterilized and flexible catheter with a 3-5 ml balloon cuff (Supa Co, Iran). The head and neck of the calf extended to facilitate the passage of the sterile BAL catheter. The BAL catheter introduced into the trachea via a tracheal tube, and its positioning confirmed by repeated coughing. The balloon cuff then inflated with 3 ml of air and subsequently, 5 aliquots of 200-300 ml pre-warmed sterile saline solution (37ºC) infused, and immediately after infusion, the lavage fluid aspirated by applying negative pressure (Danlois *et al*., 2000). The lavaged fluid mixed and pooled in a sterile tube maintained on ice and immediately transferred to the biochemistry laboratory. The calves were under critical care support after the BAL procedure to prevent any bronchial complications. To obtain a cell-free supernatant, BALF centrifuged at 400 g for 15 min.

Cytology

For cytological evaluation of BALF, the cells were precipitated by centrifuging and stained using Wright-Giemsa staining and direct smear preparation (Allen *et al*., 1992).

Crude Surfactant Extraction

Cell-free BALF supernatants centrifuged at 20000 g for 60 min at 4ºc, allowing separation of crude surfactant pellets (CSP). Subsequently, the supernatant was discarded, and CSP was separated into several aliquots and stored at -80 ºC for further analysis.

Calf Lung Surfactants Extract (CLSE) Analysis

The obtained pellets were re-suspended in CaCl2-NaCl (Merck, Whitehouse Station, NJ) solution, and the lipid part of calf lung surfactant was extracted by modified Bligh and Dyer method (Bligh and Dyer, 1959). The lower phase-separated and concentrated with a rotary evaporator (IKA Co, rv10 digital) and stored at -20 ºC. For triglyceride assay, the dried extract was re-dissolved in Isopropanol, vortexed and then the saponification reagent was added. After mixing and keeping on at room temperature for 5 min, the periodate solution was added. Subsequently, acetylacetone reagent was added and samples heated at 65ºC for 15 min in a water bath. Finally, the OD was read at 410 nm vs blank (Neri and Fring 1973). To evaluation of total lipid content, sulfuric acid was added to a test tube containing the sample, mixed well and heat in boiling water bath for 10 minutes. After cooling the samples, the phosphovanillin reagent was

added and mixed. Finally, tubes were incubated at 37 °C for 15 minutes, cooled in room temperature and OD was read at 540nm.

The analysis of total cholesterol was carried out by total cholesterol assay mentioned before by Loeffler and Mc Dougald. Briefly, isopropanol was added to the conical test tube containing the sample, mixed well and then put in room temperature for 5 min, later centrifuged for 5 min.

Subsequently, transferred clear supernatant to a clean test tube and FecL3.6H2O was added to each tube and after mixing, sulfuric acid was added and OD read at 550 nm using blank.

To analyze the hydrophilic proteins, the aqueous phase was collected and stored at -20 ºC. The phospholipids classes distribution was determined by thin-layer chromatography (TLC) on silica gel plates (Merck, Whitehouse Station, NJ; 60 F 254) using a mobile phase containing chloroform/methanol/2-propanol/triethylamine/H2O (Touchstone et al., 1983). The samples and standards include L-α-phosphatidylcholine from Soy (CAS No= 840054C, Avanti polar lipids Inc) and 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (CAS No=850355C, Avanti polar lipids Inc) and 1-stearoyl-2-linoleoyl-sn-glycero-3-phosphocholine (CAS No= 850468C, Avanti polar lipids Inc) were placed on plates and after drying, the TLC plate was put in the mobile phase containing tank. Then, the samples were stained with 10%H2SO4 and 8% H3PO4and incubated

at 150° C (oven) for 15 minutes. Finally, the phospholipids were determined by Power Scan 2017 software (Mokra *et al*., 2016).

The phospholipid contents of the extracted samples confirmed using high-performance liquid chromatography with C8 reversed-phase column 150 X 4.6 mm 5 µm (Waters) along with refractive index detection (RID). The mobile phase prepared by combining 500ml acetonitrile, 450 ml methanol and 100 ml 50 mM acetic acid to a final ratio of 50:45:10. The mobile phase was degassed for 10 minutes. The extracted samples injected into the column containing a mobile phase of acetonitrile, methanol and acetic acid (50:45:10) at the flow rate of 1 ml/min.

The total protein of the surfactant measured by the Micro-Bradford method (Bradford, 1976). Besides, SP-A, B, C and D contents analyzed using enzyme-linked immune sorbent assay (ELISA) technique by commercial kits (CAS No: E0890b, U1622b and U1623b and E1039b).

For *in situ* evaluation of extracted lung surfactant, a profile analysis tensiometer method used (PAT1, Sin- interface Technology, Germany). Measurements of dynamic interfacial tension and dilatational viscoelasticity at the water-lipid interface performed by the drop profile analysis tensiometer (Vatanparast *et al*., 2017). Briefly; the process of measurement is based on image achievement of drop profile calculated by the Gauss-Laplace equation in which all the experiments are conducted at 25ºC and atmospheric pressure.

Parameter	No.	Ave ± SEM	Normal Range

Data Analysis

Statistical analysis was conducted using SPSS software (version 21). Significance levels were set at the $p<0.05$ using One-Way ANOVA and independent t-test. All experimental procedures involving animals were approved by the Ethics Committee of the Faculty of Veterinary Medicine of Tehran University.

3.Results

Clinical Parameters and BALF Cytology

As the data in Table 1 show, in all groups, general hematological parameters were recorded in the normal range. The data showed no significant difference between cytological content of BALF at different age and sex (Table 1 and 2) ($P>0.05$).

Phospholipids and Protein Contents

It was construed from the results that sex and age had no effect on SP-A, SP-B, SP-D and total proteins contents of extracted surfactant ($P > 0.05$). However, BALF in female groups was significantly enriched by SP-C in comparison to male groups at the same age ($P=0.003$). On the other hand, the amount of SP-C was considerably increased in group A in the females in

comparison to the similar sex in other groups. Furthermore, SP-C decreased in the males of group C in comparison with the males in group A (P=0.04).

In addition, a decreasing trend by aging was observed in the amount of SP-C and SP-D in both female and male groups. As Fig. 1 shows, phospholipids composition of the extracted surfactant was confirmed by TLC (Figure 1). No significant differences were found in both age and sex on the number of total cholesterols and glycerides (P>0.05).

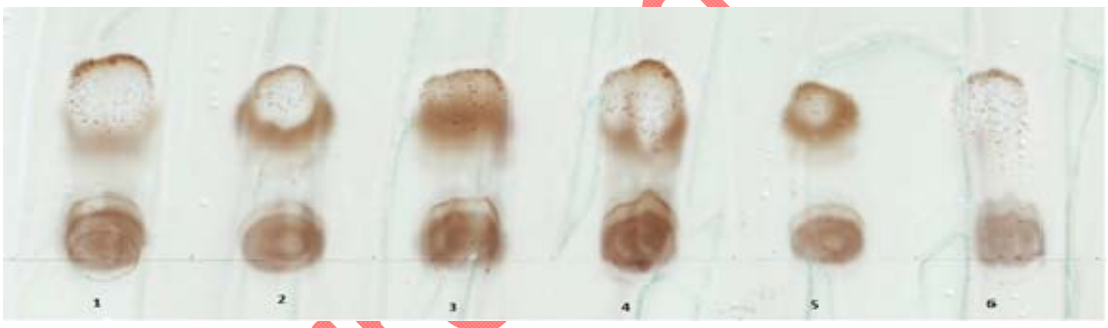

Figure 1. Phospholipids composition of extracted surfactant on TLC

1: 1,2-dipalmitoyl-**sn**-glycero-3-phosphocholine standard; 2: Sample; 3: L –α-Phosphatidylcholine standard; 4: Sample; 5:1-stearoyl-2-linoleoyl-**sn**-glycero-3-phosphocholine standard; 6: Sample

Total phospholipids in the female groups were significantly higher than the male groups in the same age group (P=0.002). Additionally, total lipids in group A were significantly higher than the other groups for both males and females (P=0.003) (Table 3). It can be inferred from the present data, all measured factors except total glycerides have a descending trend by aging in both sexes.

Surface Tension Measurement Results

Based on the obtained results, the surface tension of male samples was recorded 44.76 ± 0.38 mN/m in 0-4 months, 45.97 ± 1.25 mN/m in 4-8 months and 55.06 ± 0.45 mN/m in 8-12 months (Fig. 2). It was construed from the results that the surface tension of female samples was recorded 24. 85 ± 1.30 mN/m in 0-4 months, 25.02 ± 1.05 mN/m in 4-8 months and 50.36 ± 0.8 mN/m in 8-12 months (Figs. 3, 4, and 5).

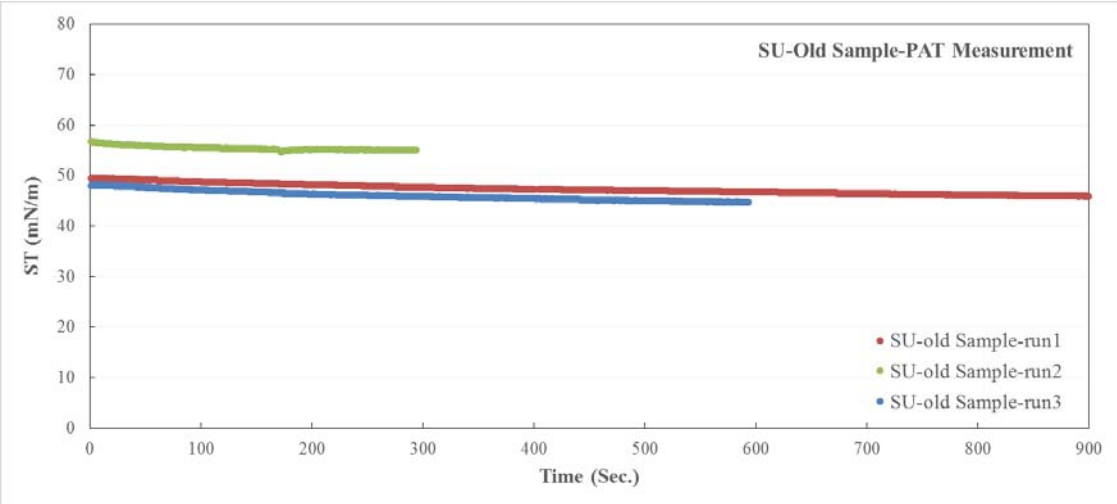

Fig 2. PAT Measurement of male samples in different ages.

Run.1: 4-8 month in male group, run.2: 0-4 month in male group, Run.2: 8-12 month in male group.

Based on PAT results, the surface tension of male samples was recorded 44.76 ± 0.38 mN/m in 0-4 months, 45.97 ± 1.25 mN/m in 4-8 months and 55.06 ± 0.45 mN/m in 8-12 months.

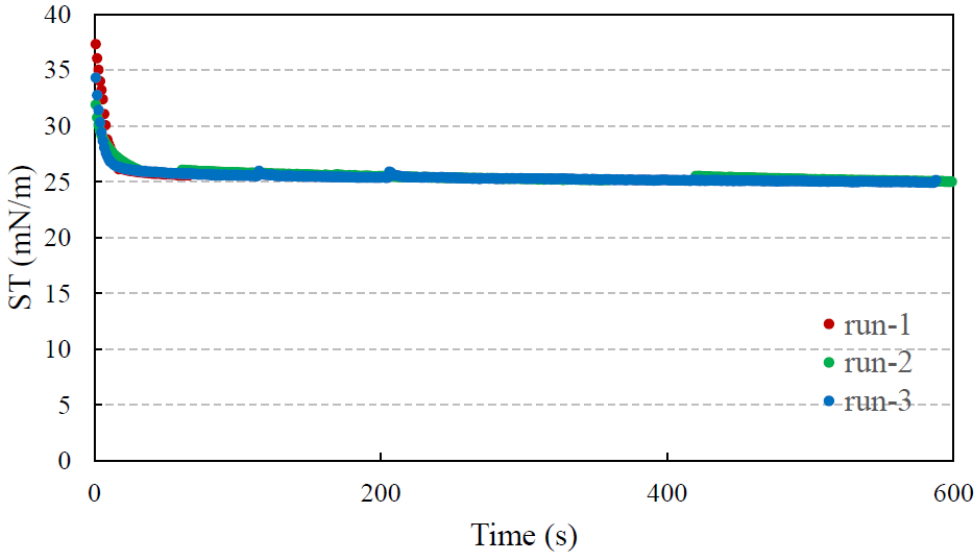

Fig 3. PAT Measurement of female samples in 0-4 months.

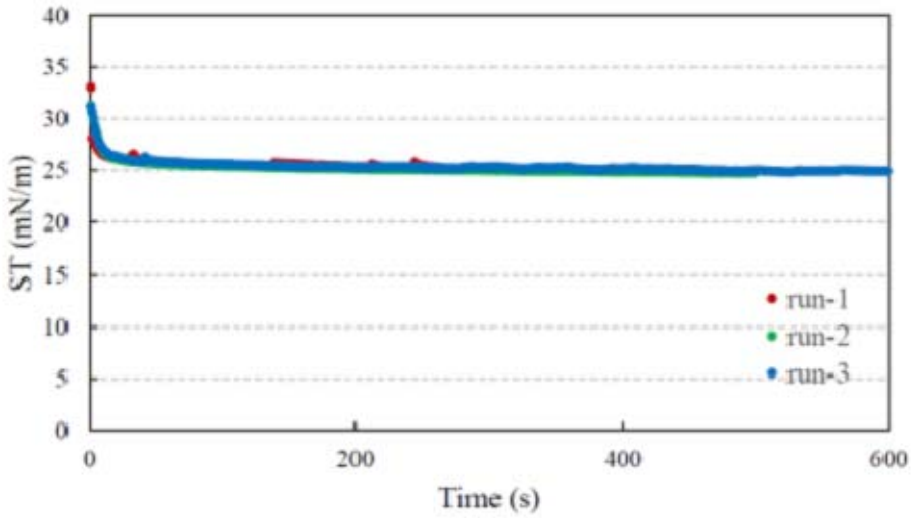

Fig 4. PAT Measurement of female samples in 4-8 months.

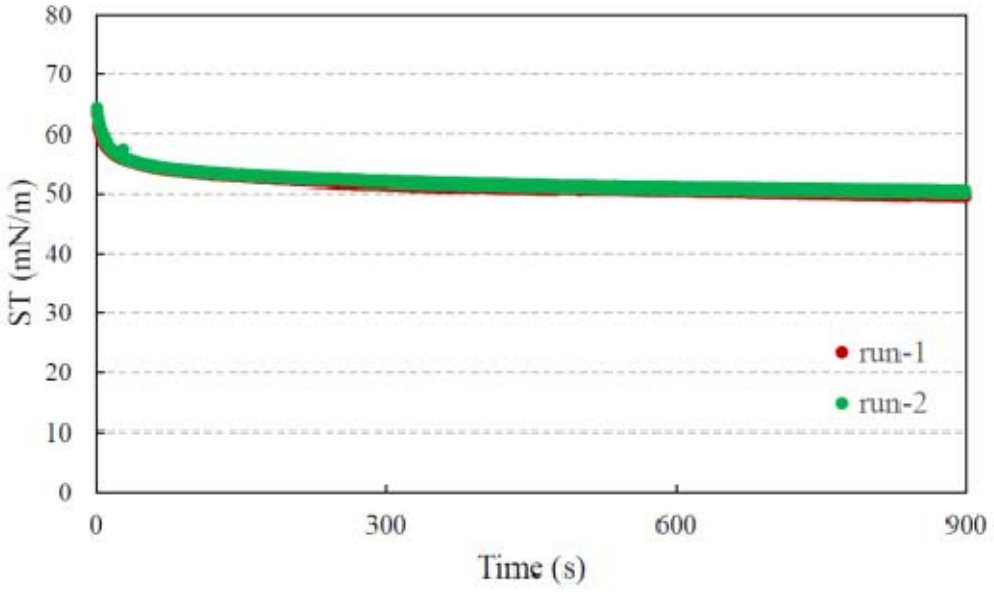

Fig 5.PAT Measurement of female samples in 8-12 months.

4.Discussion

Lung surfactant is a complex of lipoproteins that produced, stored and secreted in the type II epithelial cells, and covered the lung alveolar epithelial surface in the last weeks of pregnancy. It reduces the surface tension at the air–water interface, improves alveolar ventilation, exchanges of respiratory gases, prevents pulmonary edema formation and finally prevents the alveoli from collapsing (Khawar and Marwaha ,2023; Singh *et al*., 2021). The qualitative and quantitative of

lung surfactant change due to infectious disease and noninfectious diseases. One of the more significant findings emerged from this study is that the quality of isolated lung surfactant content in female calves less than 4 months age is higher than other groups, which can be potentially used as the best source to obtain natural surfactant to prescribe for respiratory diseases. In the other hand, it probably rationalizes the high incidence of infectious diseases such as RDS due to viral and bacterial pneumonia in the elderly because of reduce in quantity and functionality of natural lung surfactant.

The analysis of BALF undertaken in this study demonstrated that SP-C, SP-D, total phospholipid, total lipid content and functionality in BALF depended on gender, and in female groups were significantly higher in comparison to the male groups. Additionally, it would be interesting that the only compositions changed by aging are SP-C and SP-D, and the other contents of surfactant were not dependent on it. It can be inferred from the PAT data that both age and sex could affect the amount of surface tension as increased by aging and decreased in the female group in comparison to the male group. It possibly reflects the high incidence and case fatality rate of infectious diseases such as COVID-19 among males in comparison to females (Jamaatia *et al*., 2020).

SP-B is an important component of surfactant substitute mixtures can alter PL membrane association, enhancing the surfactant-like properties and the uptake of PL vesicles by type II cells *in vitro* while resisting surface tension by increasing the lateral stability of the phospholipid's monolayer (Hockenberry and Wilson,2018).

Exogenous surfactant prepared from natural sources or synthetic form is mainly used for treatment of NRDS and meconium aspiration. In addition to respiratory distress syndrome, surfactant deficiency is observed in many other clinical situations in term and preterm infants and adults. So, encourage scientist to develop different methods extraction and recognize the best sources for exogenous surfactants (Han and Mallampalli , 2015; Sardesai *et al*., 2017).

Total lipids and phospholipids in the lung surfactant are responsible for the surface active function of pulmonary surfactant by substituting interfacial water molecules, and its ultimate objective is to reduce surface tension at the water- air interface(Han and Mallampalli , 2015; Cañadas *et al*., 2020).Pulmonary surfactant proteins, particularly SP-B and SP-C, show a strong affinity for interfaces and ensuing surface-active properties indicated by many researchers as key constituents in achieving the optimal dynamic and mechanical properties of surfactant membranes (Sardesai *et al*., 2017).

The study results by Torday *et al.* revealed that male infants had a higher risk for RDS in comparison to females and the reason of this phenomenon is delay in surfactant production in response to the inhibitory effect by testes-derived hormones or diminishing the response of the male to corticosteroids stimulating surfactant synthesis (Torday *et al.*, 1981). Another study showed differences in the two genders by proteomic analysis, which may be due to varying expression of specific proteins in which the level of SP-C and D in the female was higher than the male; this can be the cause of more respiratory abnormalities in males (Sardesai *et al.*, 2017; Rahmanian *et al.*, 2014). Nielsen found that testicular hormones, by blocking the lung maturation, can contribute to higher morbidity and mortality rate in male infants (Nielsen *et al.*, 1982).

Another important factor that can change the surfactant content is aging due to reduction in alveolar, alveolar-capillary and lung parenchyma surface area. An age-related decrease in surfactant in monkeys could be explained by a lower number of type II alveolar cells per unit lung volume with aging and decreased alveolar surface tension produced by surfactants (Shimura *et al.*, 1986; Dezfouli *et al.*, 2022; Vanstapel *et al.*, 2021). Furthermore, the decreases occur with alveolar septal area, and the total surface area of the lung parenchyma, which can cause a decrease in surfactant composition (Pruthi and Multani, 2012).

Christmann et al. showed that surface tension and phospholipid composition of surfactant in neonatal foals were significantly different as compared to adult horses, and this may influence the biophysical and immunologic functions of surfactants (Christmann *et al*., 2006). However, studies on the whole-lung tissue extracted surfactant from humans, rat and rhesus monkeys revealed no significant alterations in the content of disaturated phosphatidylcholine with aging (Egberts *et al*., 1987; Ghidoni *et al*., 2015).

5.Conclusion

Finally, owing to importance of surfactant replacement therapy in preterm foals, lambs, calves and babies, obtaining the best source with new method identifications to acquire the natural surfactant to produce exogenous surfactant is necessary. Based on the obtained results, female BALF was more enriched in SP-C and total PL contents that differences in surfactant content, besides of PAT analysis, between neonate male and female may be attributed to surfactant phosphatidylcholine synthesis in late of pregnancy, but further investigation is necessary by longer age intervals.

Acknowledgments:

The authors gratefully acknowledge the financial support provided by the Institute of Biomedical Research of Veterinary Medicine, University of Tehran and Persian Darou Alborz Research and Technology Fund.

Ethical Considerations

Compliance with ethical guidelines

All animal procedures were performed following the standards outlined in the guidelines of the Animal Welfare, Ethics and Experimentation Committee of Faculty of Veterinary Science, Tehran, Iran.

Conflict of interest

The authors declared no conflict of interest.

References

Al-Abedi, G. J., Al-Eodawee, E. M., Khalili, S., & Gharban, H. A. (2022). First Molecular Genotyping of Cryptosporidium felis in Cattle, Iraq. *Archives of Razi Institute*, 77(6), 2345[DOI : 10.22092/ARI.2022.359686.2457 ; PMID: 37312738].

Allen, J.W., Viel, L., Bateman, K.G., Rosendal, S., Shewen, P.E. (1992). Cytological findings in bronchoalveolar lavage fluid from feedlot calves. Associations with Pulmonary Microbial Flora. *Canadian Journal of Veterinary Research*, 56, 122-126[PMID: 1591654].

Ashrafi, F., Azari, A. A., & Fozouni, L. (2022). Prevalence and Antibiotic Resistance Pattern of *Mannheima haemolytica* and *Pasteurella multocida* Isolated from Cattle Lung Samples from an Industrial Abattoir: A Study from Northeastern Iran. *Iranian Journal of Veterinary Medicine*, 16(4).

Bae, C. W., Kim, C. Y., Chung, S. H., & Choi, Y. S. (2019). History of pulmonary surfactant replacement therapy for neonatal respiratory distress syndrome in Korea. *Journal of Korean Medical Science*, 34(25)[DOI: 10.3346/jkms.2019.34.e175 ; PMID: 31243934].

Bligh, E AND Dyer W. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Chemistry*,37(8), 911-917[DOI: 10.1139/o59-099; PMID: 13671378].

Bradford, M. (1976). A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein-dye binding. *Analytical and Bioanalytical Chemistry*, 72, 248[DOI: 10.1006/abio.1976.9999; PMID: 942051].

Cañadas, O., Olmeda, B., Alonso, A., & Pérez-Gil, J. (2020). Lipid–protein and protein–protein interactions in the pulmonary surfactant system and their role in lung homeostasis. *International Journal of Molecular Sciences*, 21(10), 3708 [DOI: 10.3390/ijms21103708 ; PMID: 32466119].

Christmann, U., Livesey, L.C., Taintor, J.S., Waldridge, B.M., Schumacher, J., Grier, B.L., Hitem, D. (2006). Lung surfactant function and composition in neonatal foals and adult horses. *Journal of Veterinary Internal Medicine*, 20, 1402- 1407[DOI: 10.1892/0891-6640(2006)20[1402:lsfaci]2.0.co;2; PMID: 17186857].

Choi, Y., Jang, J., and Park, H. S. (2020). Pulmonary surfactants: a new therapeutic target in asthma. *Current Allergy and Asthma Reports*, 20, 1-8 [DOI: 10.1007/s11882-020-00968-8 ; PMID: 32914349].

Cutts, S., Talboys, R., Paspula, C., Prempeh, E.M., Fanous, R., Ail, D. (2017). Adult respiratory distress syndrome. *Annals of the Royal College of Surgeons of England*, 99(1): 12–16 [DOI: 10.1308/rcsbull.2017.12; PMID: 27513791]

Danlois, F., Zaltash, S., Johansson, J., Robertson, B., Haagsman, H.P., Eijek, M.V., Beers, M.F., Rollin, F., Ruysschaert, J.M., Vandenbussche, G. (2000). Very low surfactant protein C contents in newborn Belgian White and Blue calves with respiratory distress syndrome. *Biolobical Journal*,351, 779-787[DOI: 10.1042/bj3510779; PMID: 11042134].

Dezfouli, M. R. M., Zarghami, F., Rahbari, S., Abkooh, E. E., Lotfolahzadeh, S., Chaleshtori, S. S., & Shayan, P. (2022). Protection of Calves Against Cryptosporidiosis by Hyperimmunization of Pregnant Cattle Colostrum using Oocyst Whole Antigens. *Iranian Journal of Veterinary Medicine*, 16(4).

Egberts, J., Beintema ,D.A., de Boers, A.(1987).Phosphatidylinositol and not phosphatidylglycerol is the important minor phospholipid in Rhesus-monkey surfactant. *Biochimica et Biophysica Acta*, 919, 90–92[DOI: 10.1016/0005-2760(87)90221-9; PMID: 3567218].

Fozouni, L., & Tahaei, M. (2023). Anticandidal Effects of Zinc Oxide Nanoparticles on Fluconazole-Resistant Candida Isolates Causing Diarrhea in Calves, in vitro. *Archives of Razi Institute*, 78(1), 499[DOI : 10.22092/ARI.2022.359686.2457 ; PMID: 37312738].

Ghidoni, R., Caretti, A., Signorelli, P. (2015). Role of Sphingolipids in the Pathobiology of Lung Inflammation. *Mediators of Inflammation*,487-508[DOI: 10.1155/2015/487508; PMID: 26770018].

Guzmán, E., & Santini, E. (2019). Lung surfactant-particles at fluid interfaces for toxicity assessments. *Current Opinion in Colloid and Interface Science*, 39, 24-39[DOI; 10.1016/j.cocis.2019.01.003].

Han, S., & Mallampalli, R. K. (2015). The role of surfactant in lung disease and host defense against pulmonary infections. *Annals of the American Thoracic Society*, 12(5), 765-774[DOI: 10.1513/AnnalsATS.201411-507FR ; PMID: 25742123].

Hentschel, R., Bohlin, K., van Kaam, A., Fuchs, H., & Danhaive, O. (2020). Surfactant replacement therapy: from biological basis to current clinical practice. *Pediatric research*, 88(2), 176-183[DOI: 10.1038/s41390-020-0750-8 ; PMID: 31926483].

Hockenberry, M. J., & Wilson, D. (2018). Wong's nursing care of infants and children-E-book. Elsevier Health Sciences.

Jamaatia, H .,Dastana, F., Tabarsic, P., Marjanic, M., Saffaeid, A., Hashemiana, M.R.(2020). A Fourteen-day Experience with Coronavirus Disease 2019(COVID-19) Induced Acute Respiratory Distress Syndrome (ARDS): An Iranian Treatment Protocol. *Iranian Journal of Pharmaceutical Research* , 19 (1): 31-36[DOI: 10.22037/ijpr.2020.113337.14239; PMID: 32922466].

Khawar, H., & Marwaha, K. (2022). Surfactant. In StatPearls [Internet]. StatPearls Publishing [PMID: 31536207].

Magni, T., Ragni, C., Pelizzi, N., Sharma, S., Perez-Kempner, L., Turkstra, E., ... & Meshchenkova, N. (2023). Health Economic Studies of Surfactant Replacement Therapy in Neonates with Respiratory Distress Syndrome: A Systematic Literature Review. *PharmacoEconomics-Open*, 7(3), 359-371. [DOI 10.1007/s41669-023-00399-x; PMID: 36906631].

Mojibi, R., Mehrzad, J., Sharifzadeh, A., & Nikaein, D. (2022). Apoptotic Effects of Caffeic Acid Phenethyl Ester and Matricaria chamomilla Essential Oil on A549 Non-Small Cell Lung Cancer Cells. *Iranian Journal of Veterinary Medicine*, 16(4).

Mokra, D., Mikusiakova, L. T., Mikolka, P., Kosutova, P., Jurcek, M., Kolomaznik, M., & Calkovska, A. (2016). High-frequency jet ventilation against small-volume conventional mechanical ventilation in the rabbit models of neonatal acute lung injury. *Prospect in Pediatric Diseases Medicine*, 83-93 [DOI: 10.1007/5584_2016_230; PMID: 26987323].

Neri, B.P., &Fring, C.S.(1973). Improved method for determination of triglycerides in serum. *Clinical Chemistry,* 19, 1201-1203[PMID: 4355041].

Olmeda , B., Martínez, C.M., Pérez, G.J.(2017). Pulmonary surfactant metabolism in the alveolar airspace: Biogenesis, extracellular conversions, recycling. *Annals of Anatomy*, 209, 78-92[DOI: 10.1016/j.aanat.2016.09.008; PMID: 27773772].

Pruthi, N.,and Multani, N.(2012). Influence of Age on Lung Function Tests. *Journal of Experimental Social Psychology*, 8 (1),1–6 [DOI: 10.18376//2012/v8i1/67598].

Rahmanian, S.D., Wood, K.L., Lin, S., King, M.A., Horne, A., Yang, S., Wu, H.M., Diaz, P.T. (2014). Gender differences in pulmonary function, respiratory symptoms, and macrophage proteomics among HIV-Infected smokers. *Scientifica*, 1-9[DOI: 10.1155/2014/613689; PMID: 24729918].

Rawal,G., Kumar,R., Yadav, S., Sujana, R.(2017). H1N1 Influenza Induced Acute Respiratory Distress Syndrome Rescued by Extracorporeal Membrane Oxygenation: a Case Report. *Journal of Translational Internal Medicine*, 5(3): 182–185[DOI: 10.1515/jtim-2017-0018; PMID: 29085792].

Sardesai, S., Biniwale, M., Wertheimer, F., Garingo, A., Ramanathan, R. (2017). Evolution of surfactant therapy for respiratory distress syndrome: past, present, and future. *Pediatric Research*, 81(1), 240-248 [DOI: 10.1038/pr.2016.203; PMID: 27706130].

Shimura, S., Boatman, E.S., Martin, C.J. (1986). Effects of aging on the alveolar pores of Kohn andon the cytoplasmic components of alveolar type II cells in monkey lungs. *Journal of Pathology*, 148, 1-11[DOI: 10.1002/path.1711480103; PMID: 3944670].

Singh, J., Jaffe, A., Schultz, A., Selvadurai, H. (2021). Surfactant protein disorders in childhood interstitial lung disease. *European Journal of Pediatrics*, 180, 2711-2721[DOI: 10.1007/s00431-021-04066-3; PMID: 33839914].

Stichtenoth, G., Walter, G., Lange, R., Raith, M., Bernhard, W., & Herting, E. (2014). Surface tension of airway aspirates withdrawn during neonatal resuscitation reflects lung maturity. *Pediatric Pulmonology*, 49(8), 751-756 [DOI: 10.1002/ppul.22885 ; PMID: 24106079].

Torday, J.S., and Nielsen, H.C.(1981). Surfactant phospholipid ontogeny in fetal rabbit lung lavage and amniotic fluid. *Biology of the Neonate*, 39(5-6), 266-71[DOI: 10.1159/000241447; PMID: 6894869].

Torday, J.S., Nielsen, H.C., Fencl, M.M., Avery, M.E.(1981). Sex differences in fetal lung maturation. *American Review of Respiratory Disease*, 123, 205–208[DOI: 10.1164/arrd.1981.123.2.205; PMID: 6894519].

Touchstone, J.C., Levin, S.S., Dobbins, M.F., Matthews, L., Beers, P.C., Gabbe, S.G. (1983). (3-sn-Phosphatidyl)cholines (lecithins) in amniotic fluid. *Clinical Chemistry*,29, 1951–1954[DOI:10.1093/clinchem/29.11.1951].

Vatanparast, H., Javadi, A., and Bahramian, A. (2017). Silica nanoparticles cationic surfactants interaction in water-oil system. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 521, 221-230[DOI: 10.1016/j.colsurfa.2016.10.004].

Vanstapel, A., Weynand, B., Kaes, J., Neyrinck, A. P., Ceulemans, L. J., Vanaudenaerde, B. M., ... & Verleden, S. E. (2021). Interalveolar pores increase in aging and severe airway obstruction. *American Journal of Respiratory and Critical Care Medicine*, 204(7), 862-865[DOI: 10.1164/rccm.202102-0530LE ; PMID: 34241578].

اثر سن و جنس بر عملکرد سورفکتانت طبیعی ریه گوساله به عنوان یک داروی با ارزش برای درمان سندرم دیسترس تنفسی

مرضیه مخبر دزفولی[1]، زهره افتخاری*[2،4]، سیروس صادقیان چالشتری [3، 4]

1-مرکز تحقیقات قلب و عروق شهید رجایی ، مرکز آموزشی ، تحقیقاتی و درمانی قلب و عروق شهید رجایی ، دانشگاه علوم پزشکی ایران ، تهران-ایران

2-بخش بیوتکنولوژی، انستیتو پاستور ایران، تهران- ایران

3-گروه علوم بالینی، دانشکده دامپزشکی، دانشگاه تهران، تهران-ایران

4-پژوهشکده تحقیقات زیست پزشکی، دانشگاه تهران، تهران، ایران

خلاصه

زمینه ی مطالعه: سورفکتانت اگزوژن تهیه شده از منابع طبیعی به بازیابی عملکرد طبیعی ریه در نوزادان نارس کمک می کند. اختلال عملکرد سورفکتانت ریوی می تواند منجر به آسیب حاد ریه شود که معمولا با بی ثباتی آلوئول ها ، شناور شدن و کلاپس مشخص می شود. نشان داده شده است که این ناهنجاری ها در سندرم زجر تنفسی حاد (ARDS) و سندرم دیسترس تنفسی نوزادان (NRDS) رخ می دهند.

هدف: شناسایی بهترین منبع سورفکتانت طبیعی اگزوژن و ترکیب آن.

روش کار: بیست و چهار گوساله هلشتاین سالم در سه گروه سنی در هر دو جنس برای بررسی تأثیر جنسیت و سن بر ترکیب سورفکتانت انتخاب شدند. مایعات رویی بدون سلول تهیه شده از لاواژ ریه ی گوساله ها به مدت 60 دقیقه در20000 g سانتریفیوژ شدند و رسوب سورفکتانت خام (CSP) به دست آمد. پس از آن، مایع رویی دور ریخته شد و CSP به چند بخش جدا شد و برای تجزیه و تحلیل بیشتر در دمای 80- درجه سانتیگراد نگهداری شد.

نتایج: نتیجه‌گیری شد که محتوای SP-C و SP-D در گروه‌های ماده به‌طور معنی‌داری با در مقایسه با گروه‌های نر در همان سن بیشتر بود. محتوای کل فسفولیپیدها، گلیسریدها و کلسترول ها در گروه های نر وابسته به سن گزارش نشد. با این حال، محتوای این مواد با افزایش سن در گروه های ماده ، روند کاهشی را نشان داد.

نتیجه‌گیری نهایی: می‌توان چنین استنباط کرد که هم سن و هم جنس می‌توانند بر میزان کشش سطحی اثر گذار باشد به طوری که با افزایش سن میزان کشش سطحی افزایش یافته و در گروه ماده نسبت به گروه نر کاهش یافت. گوساله‌های ماده در گروه سنی جوان‌تر بهترین منبع سورفکتانت‌های طبیعی مورد نیاز برای سورفکتانت اگزوژن در بیماری‌های تنفسی نوزادان به دلیل بالاترین غلظت دی پالمیتوئیل فسفاتیدیل کولین و کمترین کشش سطحی هستند.

کلیدواژه: تکنیک تجزیه و تحلیل مشخصات، دیسترس تنفسی گوساله، ریه، فاکتور